Visualizing wound healing in fish

Ljungqvist, Martin Georg; Schmidt, Jacob; Ersbøll, Bjarne Kjær; Frosch, Stina; Nielsen, Michael Engelbrecht

Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

DTU Food
National Food Institute

DTU Informatics
Department of Informatics and Mathematical Modeling

Visualizing wound healing in fish

Martin Ljungqvist†, Jacob Schmidt*, Bjarne Kjær Ersbøll†, Stina Frosch* and Michael Engelbrecht Nielsen*

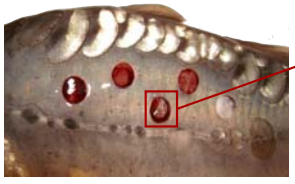
* Technical University of Denmark, National Food Institute, † Technical University of Denmark, Department of Informatics and Mathematic Modelling

The aim of the project is to create a fish wound model that can be used to quantify wound healing, and thus to assess the effect of *e.g.* feed components and bacteria on wound healing. The model will be based on image analysis of the wound as well as gene expression data from tissue samples.

Background: Dermal damage triggers a cascade of reactions aiming at repairing the damaged site, and keeping any pathogenic organisms from entering the body. The first step is the rushing of blood to the damaged area. The blood clots and thus stops excessive bleeding and serves as a matrix for the infiltration of various cell types acting in concert to kill any intruding organisms, clean up the wound, and create new tissue. During this process of wound healing, a cascade of different proteins are expressed by cells at the wound site.

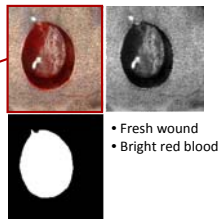
Procedure: Dermal damage is inflicted using a biopsy punch. This creates wounds of a well-defined area. Several times during the course of wound healing, the fish are anaesthetized, standardized images are aquired and tissue samples collected from the wound site.

Image analysis results from a pilot experiment on carp *Cyprinus carpio*:

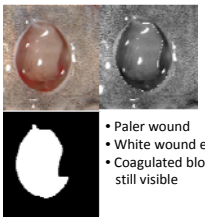


Damage is inflicted with a biopsy punch

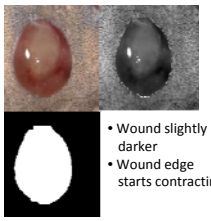
Chart: *The wound area corresponds to the number of white pixels (x10⁴) in the binary images on the right. As seen, there are some issues with the technique still to be addressed.*



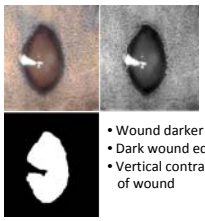
- Fresh wound
- Bright red blood



- Paler wound
- White wound edge
- Coagulated blood still visible



- Wound slightly darker
- Wound edge starts contracting

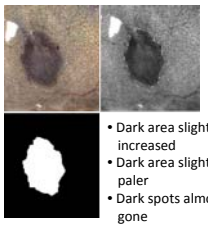


- Wound darker still
- Dark wound edge
- Vertical contraction of wound

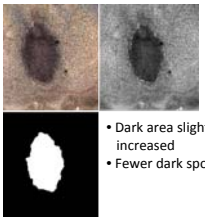
+1 minute — *+2 days* — *+1 week* — *+2 weeks*

Progression of wound healing

+9 weeks — *+7 weeks* — *+5 weeks* — *+3 weeks*



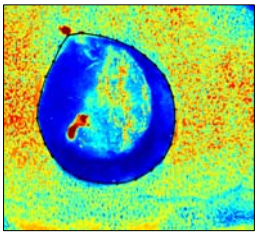
- Dark area slightly increased
- Dark area slightly paler
- Dark spots almost gone



- Dark area slightly increased
- Fewer dark spots

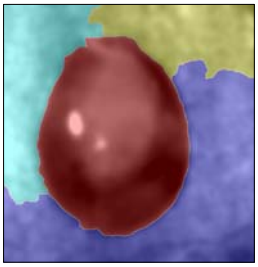
- Wound closed
- Dark spots at and adjacent to the wound site
- Dark blotch covers wound

- Wound almost closed
- Wide dark wound edge
- Pale center



Active Contours

As a starting point a circular snake with a large radius was placed in the middle of the image, the snake then shrinks to fit the nearest salient contour. The snake contour localizes the wound in this manner. The image forces were calculated using the image gradient. This method has potential but it is a challenge to make it robust for the image sequence.



Watershed Segmentation

Watershed uses the Fernand Meyer algorithm. The gradient magnitude was used as the initial segmentation function for marking borders of objects. Foreground markers were done using a combination of morphological openings, closings and reconstructions. The background markers were calculated using the intensity threshold. The most centered segment in each image represents the wound and these are used for the wound area images above.

Acknowledgement: The work presented has received funding from BioMar A/S and the EU under the Seventh Framework Programme *FP7/2007-2013* under *grant agreement* n° 214505.10